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ORIGINAL PAPER

Leucocyte profiles and corticosterone in chicks of southern rockhopper penguins

Nina Dehnhard · Maud Poisbleau · Laurent Demongin · Olivier Chastel · Hendrika J. van Noordwijk · Petra Quillfeldt

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Abstract The immune system is essential for health and survival of vertebrates, yet still little is known about the ontogeny of the immune system in wild birds. The southern rockhopper penguin (*Eudyptes chrysocome chrysocome*) is a semi-altricial seabird with a long developmental period and reversed hatching asynchrony, favouring the survival of B-chicks. We compared leucocyte counts and baseline corticosterone levels of southern rockhopper penguin chicks under different preconditions such as sex and origin from an A- or B-egg from 4 to 51 days of age. We conducted an experiment to compare leucocyte profiles and baseline corticosterone levels in A- and B-chicks in single-egg clutches as well as in B-chicks from normal two egg-clutches (one A- and one B-egg). None of these treatments influenced leucocyte counts or corticosterone levels, indicating a similar investment in the immune system. Our main finding was an increase of leucocytes/10,000 erythrocytes with age, which was mainly caused by an increase in lymphocyte numbers. This suggests differential investment into acquired immunity at this stage of development. As the granulocyte/lymphocyte (G/L) ratio did not change with age or body condition, G/L ratios seem not to reflect stress caused by poor provisioning of penguin chicks. This was also reinforced by the decrease of plasma corticosterone levels with age. Body condition was negatively correlated with monocyte numbers, suggesting a poorer health status of penguin chicks in poorer body condition. Yet, there was no link between body condition and other leucocyte parameters, indicating that chicks in a good body condition did not additionally invest into their immune system.

Keywords Leucocyte counts · G/L ratio · Baseline corticosterone · Immune system · Body condition · Southern rockhopper penguin

Introduction

The immune system as the defence mechanism against pathogens and parasites is essential for survival in vertebrates, but it is also energetically costly (Sheldon and Verhulst 1996; Norris and Evans 2000; Lee 2006). Therefore, juveniles have to trade off the investment in the immune system with growth. Leucocyte profiles have been used to gain insight into the immune system and its development with age (Quillfeldt et al. 2008) and body condition (Masello et al. 2009). Yet, data on the immune system of free-living birds are still scarce and so far we know little about its ontogeny. Besides body condition, sex and hatching order can also influence the immune system (Müller et al. 2004; Parejo et al. 2007). Moreover, the type of development (altricial, precocial, or semi-altricial) might also have a considerable effect on the development of the

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N. Dehnhard (✉) · M. Poisbleau · L. Demongin · H. J. van Noordwijk · P. Quillfeldt
Max Planck Institute for Ornithology, Vogelwarte Radolfzell, Schlossallee 2, 78315 Radolfzell, Germany
e-mail: dehnhard@orn.mpg.de

M. Poisbleau · L. Demongin
Department of Biology-Ethology, University of Antwerp, Campus Drie Eiken, Universiteitsplein 1, 2610 Antwerp (Wilrijk), Belgium

O. Chastel
Centre d'Études Biologiques de Chizé, CNRS UPR 1934, 79360 Beauvoir-sur-Niort, France

immune system, as altricial chicks are likely to get in contact with a range of pathogens much earlier than precocial ones. This should be especially true for those species that breed in colonies, as the transmission of parasites is facilitated due to the high density of birds (e.g. Brown and Brown 1986; Tella 2002).

The two most common types of leucocytes are lymphocytes (L) that belong to the acquired immunity, and heterophils (H), which form the majority of granulocytes and represent the innate immunity (Roitt et al. 1993). Limited energetic resources may limit the degree, to which an individual can invest in acquired immunity (Lochmiller and Deerenberg 2000). Thus, the ratio of these two fractions, the heterophil/lymphocyte (H/L) or the granulocyte/lymphocyte (G/L) ratio, has been described as a measurement of stress in birds (Gross and Siegel 1983; Ots and Hõrak 1996; Hoi-Leitner et al. 2001; Davis et al. 2008). The H/L ratio has moreover been related to baseline corticosterone levels in adult birds (e.g. Davis et al. 2008). The steroid hormone corticosterone is released from the hypothalamic–pituitary–adrenal axis under acute stress (Maxwell 1993). During chronic stress, such as under poor feeding conditions, plasma baseline corticosterone levels remain elevated and lead to adaptive changes in physiology and behaviour, including an increase in the H/L ratio (e.g. Maxwell 1993; Maxwell and Robertson 1998; Davis et al. 2008). Therefore, one could expect to find a connection between these two parameters in chicks as well, provided that the H/L ratio in chicks reflects chronically stressful conditions rather than the ontogeny of the immune system.

Here, we studied a semi-altricial seabird, the southern rockhopper penguin (*Eudyptes chrysocome chrysocome*). In this species, although two eggs are laid (first the smaller A-, and then the larger B-egg), usually only the chick from the B-egg (B-chick) is reared by the parents (Williams 1995). After hatching, chicks are guarded by the male for about 3 weeks, while the female forages and feeds the chick (Strange 1982). Then, chicks start to form crèches, at which point both parents feed them (Williams 1995). We manipulated nests and artificially created broods with only one A- or B-egg next to the normal two-egg clutches (Poisbleau et al. 2008). These A- and B-chicks were sampled during guard and crèche to obtain leucocyte counts and corticosterone levels at different ages.

Materials and methods

Study area and sampling procedures

Field work was conducted in the "Settlement Colony" on New Island, Falkland Islands (51°43 S, 61°17 W) during the breeding season 2006–2007. Nests were monitored daily, and randomly assigned to a treatment: A-nest, B-nest or AB-nest. To obtain obligate A- or B-egg clutches, we removed one egg when the clutch was complete, so that A-nests held only the A-egg and B-nests only the B-egg, while AB-nests contained both eggs. The removed eggs were conserved for later hormone analyses (Poisbleau et al. 2009a, b). 45 individual chicks (13 A-chicks from A-nests, 13 B-chicks from B-nests, 19 B-chicks from AB-nests) were captured up to two times during the guard stage ($N = 35$ and 26; aged 4–10 and 11–17 days, respectively) and once during crèche ($N = 44$; aged 25–51 days), resulting in three age groups. Due to the low survival of A-chicks from AB-nests (Poisbleau et al. 2008), we did not include this treatment group into our study. Chicks from two-egg clutches were marked with non-toxic paint marker when they were less than 5 days old, and again with coloured and numbered Velcro bands on each flipper when they reached 5 days of age (Poisbleau et al. 2008).

Blood samples were taken from the brachial vein (1 ml heparinised syringe, 25 gauge needle) immediately within 3 min after capture. We measured bill length (exposed culmen) to the nearest 0.1 mm using callipers and flipper length (extended from axilla) with a ruler to the nearest millimetre (Poisbleau et al. 2008). We weighed chicks with a body mass less than 300 g to the nearest gram using a digital balance and heavier chicks were weighed to the nearest 10–20 g using an adapted spring balance (Poisbleau et al. 2008). We followed Ruiz et al. (2002) in accomplishing blood smears. These were later fixed with methanol (100%) and stained with Giemsa.

Leucocyte counts, hormone and sex analysis

Blood smears were scanned with a light microscope (1,000×, oil immersion) for a monolayer of blood cells. Differential leucocyte counts were accomplished along the short-axis of the slide to control for differences in thickness of blood cells, following previous authors (e.g. Merino et al. 1999, Masello et al. 2009). A minimum of 100 leucocytes were counted per slide and distinguished as granulocytes (heterophils, eosinophils, and basophils pooled together), lymphocytes, and monocytes following the criteria of Hawkey and Dennet (1989). Relative numbers of each leucocyte types were calculated as the percentage of all leucocytes ("relative leucocyte numbers"). Leucocyte numbers per 10,000 erythrocytes were calculated by counting the number of all erythrocytes in one microscopic visual field and multiplying it with the number of the microscopic visual fields that were scanned until reaching 100 leucocytes, following Lobato et al. (2005). Thus, we gained the following immunological parameters: G/L ratio, granulocytes (%), lymphocytes (%), monocytes (%), granulocytes/10,000 erythrocytes, lymphocytes/10,000 erythrocytes,

monocytes/10,000 erythrocytes, and total leucocytes/10,000 erythrocytes.

Corticosterone concentrations (ng/ml) were determined at CEBC in Chizé following the procedure detailed in Lormée et al. (2003). All samples were run in one assay. The intra-assay coefficient of variation assessed using three reference plasmas was 4.91% ($n = 9$ duplicates). The lowest detectable concentration was 0.2 ng/ml while the lowest measurement was 0.31 ng/ml.

As sex determination from morphological measurements is not highly reliable for young chicks (Poisbleau et al. 2010), sexes of chicks were determined using DNA analyses. We extracted DNA by adding 20 µl of blood cells stored in 70% ethanol to 100 µl QuickExtract™ DNA extraction solution (Epicentre). We followed the manufacturer's protocol, but optimized the suggested incubation times to 15 min (65°C) at the first heating step and 3 min (98°C) at the second heating step. Extracted DNA was stored at −20°C. We performed the PCR with primers 2945F, cfR and 3224R according to Ellegren (1996). All samples were run on a 1.5% agarose gel and checked for a single (male) or double (female) band.

Statistics

Statistical tests were performed with SPSS 11.0. Data were tested for normality using Kolmogorov–Smirnov tests and were transformed if necessary. Even though count-data are usually transformed using square root, we received normally distributed data only with the logarithmic transformation. Data of G/L, granulocytes/10,000 erythrocytes, and leucocytes/10,000 erythrocytes were ln transformed, monocytes (%), monocytes/10,000 erythrocytes and corticosterone were ln transformed after adding 1, as they contained 0 values. As mass and measurements of structural size (flipper, bill) change with age (see Fig. 1), we did not use body mass alone to estimate the nutritional state of the chicks. Instead, we controlled the body mass for measures of structural size by calculating individual body condition (see e.g. Quillfeldt et al. 2008, Masello et al. 2009, Norte et al. 2009). We used a multiple linear regression of body mass as the dependent variable and flipper length and bill length as predictors to calculate body condition separately for male and female chicks ($N = 56$, $R = 0.951$, $F = 256.003$ and $P < 0.001$ for males, and $N = 42$, $R = 0.974$, $F = 365.997$ and $P < 0.001$ for females). Body condition was calculated as the residuals from observed and expected body mass (for males: $1.36 \times$ (flipper length) $+ 85.49 \times$ (bill length) $- 1202.86$; for females: $3.37 \times$ (flipper length) $+ 74.59 \times$ (bill length) $- 1104.36$). Thus, chicks in good body condition had positive residuals, i.e. the observed body mass was higher than the expected body mass.

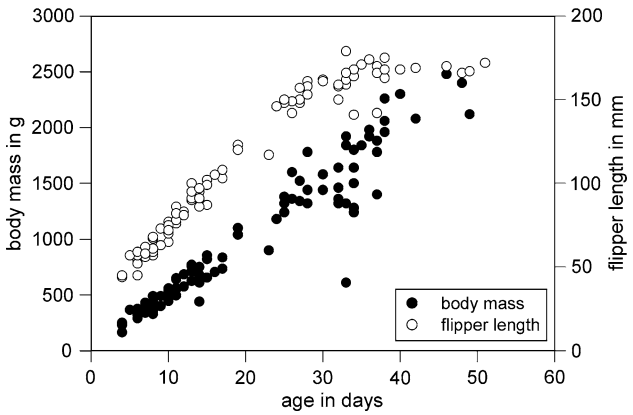

Fig. 1 Development of body mass and flipper length with age in 35 rockhopper penguin chicks. The two outliers at 14 and 33 days of age both represent one individual chick that died of starvation during crèche

General linear models (GLMs) were used to test for differences in leucocyte parameters and corticosterone (dependent variables) with age and body condition (covariates) and age and corticosterone (covariates; only for leucocyte parameters). GLMs were based on Type III Sum of Square and the results contain partial η^2 values as a measure of effect size, and a t value to indicate the direction of the relationship. Nest was originally included in the analysis to control for individual effects, as samples of some chicks were included in each age group. As this factor did not reveal a significant influence on the tested variables, nest was subsequently removed from the analyses. The removal of the factor nest did not change the results. Effects of hatching order (A- or B-chicks) and sex (male or female) were tested using independent t tests within age groups to avoid pseudo-replication. Those chicks with missing data of body condition and corticosterone were excluded from the concerned analyses in the GLMs.

In addition, we conducted repeated measures ANOVAs to test for the influence of sex and hatching order on leucocyte parameters and corticosterone (taken as dependent variables). Hatching order and sex were included as factors and the serial samplings of the same bird at different ages were included as repeated measures. This statistic allows control for the effects of sex and hatching order on age, and avoids the use of independent t tests. However, as only 18 chicks were sampled three times, we had a reduced sample size for the repeated measures ANOVAs. Therefore, we present both the results of the GLMs and the repeated measures ANOVAs.

Table 1 Mean values of differential leucocyte counts and granulocyte/lymphocyte ratio (G/L ratio) of southern rockhopper penguin chicks ($N = 105$)

	Mean ± SE	Range
G/L ratio	0.67 ± 0.03	0.1–1.7
Granulocytes (%)	35.95 ± 0.99	10.0–57.8
Lymphocytes (%)	58.50 ± 0.98	33.0–86.0
Monocytes (%)	5.61 ± 0.34	0.0–17.5
Granulocytes/10,000 erythrocytes	28.20 ± 1.65	4.4–102.2
Lymphocytes/10,000 erythrocytes	42.92 ± 1.44	12.7–76.2
Monocytes/10,000 erythrocytes	4.32 ± 0.33	0.0–17.0
Leucocytes/10,000 erythrocytes	75.43 ± 2.90	27.0–176.7

Results

Lymphocytes were the most abundant leucocyte in southern rockhopper penguin chicks (Table 1), resulting in a mean G/L ratio lower than one. Monocytes were less frequent or even totally absent.

Leucocyte counts in relation to body condition, age, sex, and hatching order

We found that the ratios of lymphocytes/10,000 erythrocytes and total leucocytes/10,000 erythrocytes were positively correlated with age, but not with body condition (Tables 2, 3; Fig. 1). Relative lymphocyte numbers, granulocytes, and G/L ratios were not related to age or body condition (Tables 2, 3). Relative monocyte numbers and monocytes/10,000 erythrocytes were negatively correlated with body condition, but not with age (Tables 2, 3). None of the leucocyte parameters were related to plasma corticosterone levels (GLMs with leucocyte parameters as dependent variables, and age and corticosterone as covariates, all $P > 0.27$ for corticosterone effect).

Leucocyte numbers except for monocytes, did not differ between male and female chicks (t tests within age groups, all $P > 0.100$). Relative monocyte numbers and monocytes/10,000 erythrocytes were slightly elevated in female chicks compared to male chicks during late guard (11–17 days old) and crèche (25–51 days old) (Fig. 1); however, statistical tests revealed no significance (t tests within age groups, $0.080 \geq P \geq 0.101$). This trend for sexual differences in relative monocyte numbers was also found in the repeated measures ANOVA (Table 3).

There were no differences in immunological parameters between A- and B-chicks (t tests within age groups, all $P > 0.145$), nor were there differences in B-chicks from B-nests and AB-nests (t tests within age groups, all $P > 0.252$).

Corticosterone levels in relation to body condition, age, sex, and hatching order

Baseline plasma corticosterone levels were negatively associated with age, but not with body condition (Table 2; Fig. 2; Spearman Rho = −0.267, $P = 0.008$ for corticosterone with age). Female chicks had higher corticosterone levels during the guard period than male chicks, but this trend was not significant (t tests within age groups, $P = 0.068$ and 0.065 for chicks aged 4–10 and 11–17 days, respectively; results of repeated measures ANOVA also not significant; Table 3; but see Figs. 2, 3). During crèche, male and female chicks did not differ in corticosterone levels ($P = 0.776$).

Table 2 Effect of age and body condition on leucocyte parameters and corticosterone concentrations of rockhopper penguin chicks

Parameter	Age	Body condition
G/L ratio[a]	$F = 0.75$, $P = 0.388$, $t = -0.87$, $\eta^2 = 0.008$	$F = 2.69$, $P = 0.104$, $t = 1.64$, $\eta^2 = 0.027$
Granulocytes (%)	$F = 0.37$, $P = 0.543$, $t = -0.61$, $\eta^2 = 0.004$	$F = 2.97$, $P = 0.088$, $t = 1.72$, $\eta^2 = 0.030$
Lymphocytes (%)	$F = 1.04$, $P = 0.310$, $t = 1.02$, $\eta^2 = 0.011$	$F = 0.76$, $P = 0.386$, $t = -0.87$, $\eta^2 = 0.008$
Monocytes (%)[a]	$F = 2.91$, $P = 0.091$, $t = -1.71$, $\eta^2 = 0.029$	$F = 8.00$, $\mathbf{P = 0.006}$, $t = -2.83$, $\eta^2 = 0.076$
Granulocytes/10,000 ery[a]	$F = 2.66$, $P = 0.106$, $t = 1.63$, $\eta^2 = 0.027$	$F = 3.15$, $P = 0.079$, $t = 1.77$, $\eta^2 = 0.031$
Lymphocytes/10,000 ery	$F = 15.39$, $\mathbf{P < 0.001}$, $t = 3.92$, $\eta^2 = 0.137$	$F = 0.35$, $P = 0.553$, $t = 0.60$, $\eta^2 = 0.004$
Monocytes/10,000 ery[a]	$F < 0.01$, $P = 0.982$, $t = 0.02$, $\eta^2 < 0.001$	$F = 4.20$, $\mathbf{P = 0.043}$, $t = -2.05$, $\eta^2 = 0.042$
Total leucocytes/10,000 ery[a]	$F = 9.19$, $\mathbf{P = 0.003}$, $t = 3.03$, $\eta^2 = 0.087$	$F = 0.57$, $P = 0.452$, $t = 0.76$, $\eta^2 = 0.006$
Corticosterone[a]	$F = 11.86$, $\mathbf{P = 0.001}$, $t = -3.44$, $\eta^2 = 0.114$	$F = 2.44$, $P = 0.122$, $t = 1.56$, $\eta^2 = 0.026$

General linear models were carried out for each leucocyte parameter and corticosterone concentrations (as dependent variable) separately, with age and body condition as covariates. The degree of freedom was 1 for every test, the sample size (N) was 100. F gives the F value, η^2 serves a measure of the effect size, and the t value indicates the direction of the relationship. Ery stands for erythrocytes. Statistically significant effects are marked bold

[a] Transformed data

Table 3 Results of repeated measures ANOVA for leucocyte parameters and corticosterone concentrations (dependent variable), with hatching order (A- or B-chick) and sex (male or female) as factors and different ages as repeated measures

Parameter	Dependent variable	Hatching order	Sex
G/L ratio[a]	$F = 0.189$, $P = 0.830$, $\eta^2 = 0.028$	$F = 0.710$, $P = 0.414$, $\eta^2 = 0.048$	$F = 0.611$, $P = 0.447$, $\eta^2 = 0.042$
Granulocytes (%)	$F = 0.197$, $P = 0.823$, $\eta^2 = 0.029$	$F = 0.720$, $P = 0.410$, $\eta^2 = 0.049$	$F = 0.237$ $P = 0.634$, $\eta^2 = 0.017$
Lymphocytes (%)	$F = 0.185$, $P = 0.834$, $\eta^2 = 0.028$	$F = 0.877$, $P = 0.365$, $\eta^2 = 0.059$	$F = 1.026$, $P = 0.328$, $\eta^2 = 0.068$
Monocytes (%)[a]	$F = 0.223$, $P = 0.803$, $\eta^2 = 0.033$	$F = 0.607$, $P = 0.449$, $\eta^2 = 0.042$	$F = 4.676$, $\mathbf{P = 0.048}$, $\eta^2 = 0.250$
Granulocytes/10,000 ery[a]	$F = 0.3019$, $P = 0.084$, $\eta^2 = 0.317$	$F = 0.053$, $P = 0.822$, $\eta^2 = 0.004$	$F = 1.170$, $P = 0.298$, $\eta^2 = 0.077$
Lymphocytes/10,000 ery	$F = 5.265$, $\mathbf{P = 0.021}$, $\eta^2 = 0.448$	$F = 0.189$, $P = 0.670$, $\eta^2 = 0.013$	$F = 0.475$, $P = 0.502$, $\eta^2 = 0.033$
Monocytes/10,000 ery[a]	$F = 2.142$, $P = 0.157$, $\eta^2 = 0.248$	$F = 0.201$, $P = 0.661$, $\eta^2 = 0.014$	$F = 3.750$, $P = 0.073$, $\eta^2 = 0.211$
Total leucocytes/10,000 ery[a]	$F = 5.241$, $\mathbf{P = 0.021}$, $\eta^2 = 0.446$	$F = 0.005$, $P = 0.945$, $\eta^2 < 0.001$	$F = 1.042$, $P = 0.325$, $\eta^2 = 0.069$
Corticosterone[a]	$F = 1.859$, $P = 0.202$, $\eta^2 = 0.253$	$F = 1.735$, $P = 0.212$, $\eta^2 = 0.126$	$F = 0.049$, $P = 0.829$, $\eta^2 = 0.004$

$N = 18$ for leucocytes and 16 for corticosterone concentration. The degree of freedom was 2 for the dependent variable and 1 for hatching order and sex. F gives the F value, η^2 serves a measure of the effect size. Ery stands for erythrocytes. Statistically significant effects are marked bold

[a] Transformed data

The origin from an A- or B-egg did not have an influence on corticosterone levels (t tests within age groups; all $P > 0.118$; repeated measures ANOVA: $P = 0.829$), and the origin from a B-nest or AB-nest did not affect corticosterone levels of B-chicks (all $P > 0.211$).

Discussion

The main finding of the present study was a strong increase of leucocytes and lymphocytes per 10,000 erythrocytes with age. Body condition only affected monocytes, but no other leucocyte type. Corticosterone levels decreased with age and, like monocyte counts, revealed minor sexual differences.

Age

Age did not have a significant effect on the G/L ratio and relative numbers of granulocytes and lymphocytes. However, lymphocytes/10,000 erythrocytes and leucocytes/10,000 erythrocytes increased and corticosterone levels decreased with age.

Avian nestlings hatch with a low concentration of erythrocytes, and their number, together with the haematocrit, rises with age during the nestling period (Merino and Barbosa 1997; Villegas et al. 2002; Gayathri et al. 2004). We, therefore, anticipated a decrease in leucocytes/10,000 erythrocytes with age. However, we found the opposite trend. Leucocytes/10,000 erythrocytes as well as lymphocytes/10,000 erythrocytes increased significantly with age. This implies a proportionally larger increase in leucocytes than erythrocytes, which might indicate a strong investment in the immune system in this species. It is also possible that the increase in leucocytes was the result of a recent infection (e.g. Hawkey and Dennet 1989) or immunization process (e.g. Eeva et al. 2005), given that the increase was proportionally larger in lymphocytes that are part of the acquired immune system. The acquired immune system acts against various pathogens, including viruses, bacteria and ectoparasites (see e.g. Apanius 1998; Lochmiller and Deerenberg 2000; Blount et al. 2003; Pap and Márkus 2003; Lee 2006). The absence of ectoparasites in rockhopper penguin chicks is notable, although sympatrically breeding imperial shags (*Phalacrocorax atriceps*) have abundant ticks and mallophaga (personal observation). The absence of ectoparasites in penguin chicks might, therefore, be explained by a strong investment in immune defence, as our data suggest.

In contrast to thin-billed prions (*Pachyptila belcheri*; Quillfeldt et al. 2008), and red-tailed tropicbirds (*Phaeton rubricauda*; Dehnhard unpublished data), we did not find effects of age on the G/L ratio and relative granulocyte and lymphocyte counts. Although all three species are classified as semi-altricial, both thin-billed prions and red-tailed tropicbirds have long nestling developmental periods, while rockhopper penguin chicks leave their nests already around 20 days of age to form crèches. Therefore, the immune system in penguin chicks might be further developed, explaining that small chicks during guard have already the same relative proportions of heterophils and lymphocytes as larger chicks during crèche.

Investment in the immune system implies a high metabolic cost due to rapid cell proliferation (e.g. Lochmiller and Deerenberg 2000). During the breeding season 2006–2007, rockhopper penguins had high chick body masses and breeding success (Poisbleau et al. 2008), indicating favourable conditions in terms of prey availability. Baseline corticosterone levels also decreased with age, further suggesting that feeding conditions were adequate for the chicks

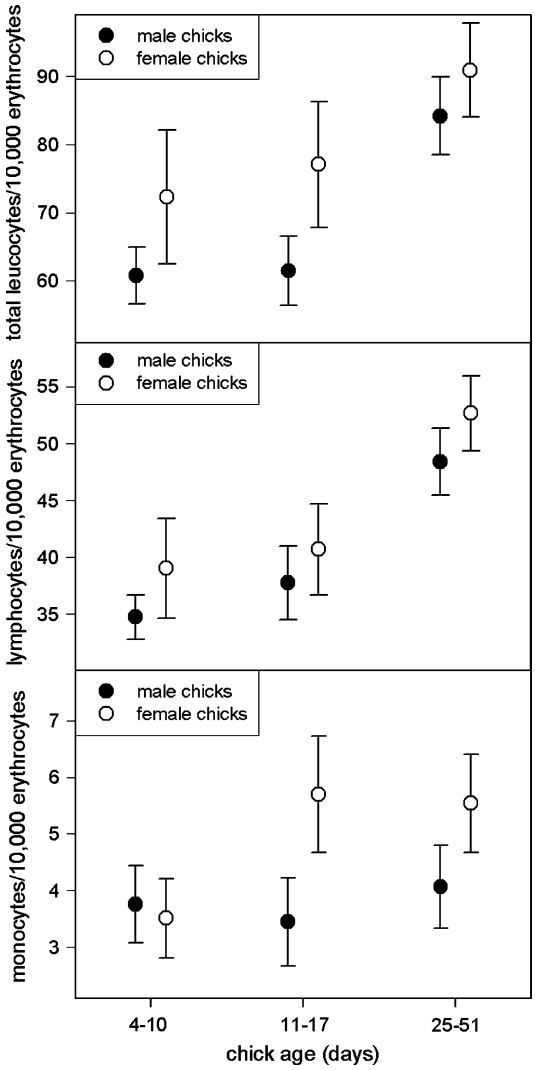

Fig. 2 Development of total leucocytes, lymphocytes and monocytes per 10,000 with age in southern rockhopper penguin chicks. Sample sizes were $N = 35$ (21 males, 14 females) for small chicks aged 4–10 days, 26 (13 males, 13 females) for medium chicks aged 11–17 days (both sampled during guard), and 44 (25 males, 19 females) for large chicks during crèche aged 25–51 days

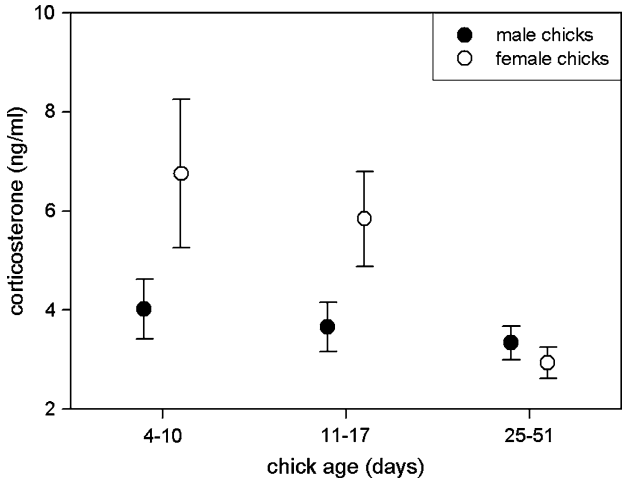

Fig. 3 Change of corticosterone levels in southern rockhopper penguin chicks with age. For sample sizes see Fig. 2

in this season. Under such favourable conditions, the trade-off between investment in innate versus acquired immunity may be minimal. Thus, G/L ratios did not change during development in this study probably because chicks were able to increase production of both granulocytes and lymphocytes in parallel.

Body condition

We did not find a correlation between body condition and the G/L ratio or any other type of leucocyte, except for monocytes. Our results are therefore in contrast to the positive correlations of body conditions with H/L ratios in nestlings of burrowing parrots (*Cyanoliseus patagonus*; Masello et al. 2009) and Eurasian treecreepers (*Certhia familiaris*; Suorsa et al. 2004), but agreed with data from chicks of other seabird species like thin-billed prions (Quillfeldt et al. 2008), and red-tailed tropicbirds (Dehnhard unpublished data). Thus, rockhopper penguin chicks in better body condition did not invest in higher granulocyte numbers (i.e. innate immunity).

We did, however, find a negative correlation between monocytes (both relative and total counts/10,000 erythrocytes) and body condition. Monocytes take part in the defence against intracellular pathogens such as bacteria and viruses (Maxwell 1993) and increase during acute inflammation (van Furth 1985); thus, high monocyte numbers might suggest poor health or some acute inflammation process. Our data, therefore, suggest that chicks in poor body condition were in a worse state of health, as they also had elevated monocyte numbers. Yet we did not find elevated G/L ratios in these individuals, which we would expect if individuals in poorer health are also facing increased stress. Animals, however, can reduce activity and therewith energy expenditure during infection (see e.g. Day and Edman 1983). Our data could indicate that some types of infection might be antagonized by monocytes exclusively, without influencing the other leucocyte types.

Moreover, we found no linkage between corticosterone and any leucocyte parameter, including the G/L ratio, and assume that G/L ratios in rockhopper penguin chicks do not reflect stress. While we acknowledge that this might be true only under good environmental conditions, our corticosterone results underline the finding that G/L ratios are chiefly related to development of leucocyte numbers with age and therewith the ontogeny of the immune system.

Differences with sex and hatching order

Sexual differences in glucocorticoid concentrations have been found in various vertebrate species, in adult and immature mammals as well as in fish and amphibians (see Manire et al. 2007 and literature therein) and birds (e.g. Lobato et al. 2008). Southern rockhopper penguin chicks have a low sexual dimorphism. Nevertheless, there was a trend for elevated monocyte numbers and corticosterone levels in female compared to male chicks. However, these sexual differences in corticosterone decreased with age and had completely disappeared during crèche. Therefore, they might be related to maternal effects (see Hayward and Wingfield 2004; Hayward et al. 2005; Love et al. 2005; Poisbleau et al. 2009b; but see Rettenbacher et al. 2009), but it seems unlikely that these pre-hatching influences were still detectable in chicks aged up to 17 days.

A- and B-chicks did not differ for leucocyte counts or corticosterone. In rockhopper penguins, A-chicks from experimental single-clutches had the same potential to survive until fledging as B-chicks, and B-chicks survived equally well in single-egg clutches compared to normal two-egg (AB-)clutches (Poisbleau et al. 2008). Our results suggest that A- and B-chicks invest similarly in the immune system when they are alone and, therefore, from their state of health, have similar chances of survival. This is also reflected in the similar corticosterone levels.

In conclusion, the analysis of leucocyte counts and corticosterone in southern rockhopper penguin chicks suggests a differential investment, particularly into acquired immunity, as evidenced by the increase of lymphocytes and leucocytes/10,000 erythrocytes with age. In addition, we could show that body condition is negatively correlated with monocyte numbers (both relative and total numbers/10,000 erythrocytes), indicating an influence of health on body condition or vice versa. The observed trend of higher corticosterone levels in female compared to male chicks during guard suggests a need for further research on yolk and chick corticosterone levels, based on the questions: Do yolk corticosterone levels differ between the sexes? Why does the difference in corticosterone levels between male and female chicks disappear with age?

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